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Confocal Imaging of Sebaceous Gland Hyperplasia In Vivo to Assess Efficacy and Mechanism of Pulsed Dye Laser Treatment

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Background and Objective: **This case demonstrates, for the first time, the use of in vivo confocal imaging to assess the efficacy of laser treatment of a skin lesion with a vascular component.**
Study Design/Patient and Method: **A patient with lesions of sebaceous gland hyperplasia was histologically imaged in vivo before and after treatment with a 585 nm pulse dye laser (PDL) by using a near-infrared, confocal reflectance microscope. Hyperplastic sebaceous ducts and sebaceous glands were seen with high resolution in vivo. Prominent dermal vasculature was viewed as well as its selective targeting by PDL.**
Conclusion: **Our results confirm the previously reported successful treatment of sebaceous gland hyperplasia with the 585 nm PDL. Lasers Surg. Med. 25:8–12, 1999.** **© 1999 Wiley-Liss, Inc.**

Key words: vascular lesion; in vivo confocal imaging; noninvasive method

REPORT OF THE CASE

A 51-year-old Caucasian woman was evaluated for asymptomatic lesions on her face and forehead, which she felt were cosmetically unsightly. On physical examination there were scattered, firm, skin colored to yellow-tinged papules, varying in size from 2–6 mm and located on the temporal and frontal forehead as well as malar regions (Fig. 1A). Some of the lesions showed central umbilication and a beaded border and a clinical diagnosis of sebaceous hyperplasia was made.

The patient was offered to participate in a study to observe these lesions using an in vivo confocal reflectance microscope (CM) before and after treatment with the 585 nm pulsed dye laser (PDL). The microscope is equipped with a low power Nd:YAG laser (1,064 nm wavelength), and allows to visualize noninvasively, in real time, the main histologic features of these lesions using 30×, 60×, and 100× objective lenses.

Therapeutical Challenge

After obtaining informed consent from the patient, two of the larger lesions were treated each with two overlapping pulses of 585 nm pulsed dye laser at 7.0 J/cm^2 using a 5 mm delivery point. Photographs and optical images by CM were taken before and after treatment. Follow-up visits at two weeks, two months, and six months revealed progressive involution (Fig. 1B). There was no evidence of pigmentary changes or scars

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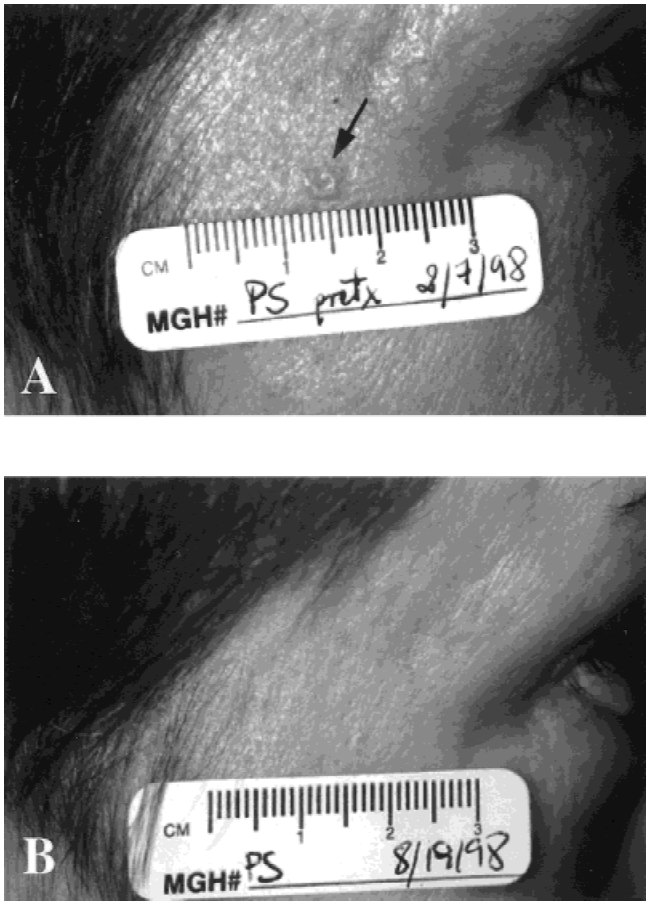

Fig. 1. **A:** A typical sebaceous hyperplasia lesion located on right temporal region before 585 nm PDL treatment. **B:** Completely involuted lesion on right temporal region at 6 months after 585 nm PDL treatment.

associated with the treatment. The patient was very satisfied with the results.

Diagnostic Challenge

After signed informed consent, the two lesions selected for treatment with a 585 nm PDL were histologically observed before and after the treatment by using CM. Pretreatment, a hyperplastic epithelium was observed at the infundibulum of the ducts with a prominent surrounding vasculature resembling a vascular crown (Fig. 2A–D). Single intravascular cells were seen, and were more apparent on active video than on still, digitized images (Fig. 2E). Dermal connective tissue was observed beginning at 85 μm below epidermis (Fig. 2C). Sebaceous gland lobules were observed at 180 μm below the stratum corneum (Fig. 2F) circumscribed within the vascular envelope. Sebocytes appeared in confocal images with a dark central nucleus surrounded by a multilobular bright cytoplasm, the latter probably due to the reflected light from lipid droplets. Confocal images obtained immediately after treatment showed the selective photothermal damage confined to the blood vessels (Fig. 3A). Two-week and two-month follow-up showed a nonhyperplastic epithelial duct with smoother contours (Fig. 3B–D). Deeper penetration images showed dense collagen bundles without dilated dermal blood vessels (Fig. 3E,F).

DISCUSSION

Sebaceous gland hyperplasia is a common lesion in cosmetic dermatology. In most of the cases, diagnosis can be established on clinical examination, but because of their resemblance to basal cell epithelioma and other hamartomas, histological evaluation is sometimes required.

This case report supports a previous clinical study [1] that reported more than 40 patients with sebaceous hyperplasia treated with the 585 nm PDL. Good cosmetic results were obtained with two or three consecutive sessions at fluences 6.5–8 J/cm^2. However, the mechanism by which 585 nm PDL induced complete disappearance of the hyperplastic sebaceous glands was not addressed. Similarly, our patient responded to one to three consecutive sessions of overlapped pulses of energy density 7–8 J/cm^2, and no textural changes were observed.

This case also demonstrates, for the first time, the use of in vivo confocal imaging to assess the efficacy of laser treatment of a skin lesion with a vascular component. It was possible to visualize the dynamic changes that occur after laser therapy that otherwise could not have been appreciated using conventional histology.

CM is an optical imaging tool that allows sectioning in vivo with high spatial resolution and contrast [2–4]. A confocal microscope is composed of a point light source (collimated laser beam) that illuminates a small area within the tissue. Detection is through a small aperture (pinhole), which is optically conjugate to the illuminated area. Unlike standard optical techniques such as conventional histology or confocal fluorescence microscopy, imaging can be done in real time and noninvasively (without excisional biopsy, tissue processing, or exogenous dyes). Therefore, skin morphology (including dynamic processes) may be

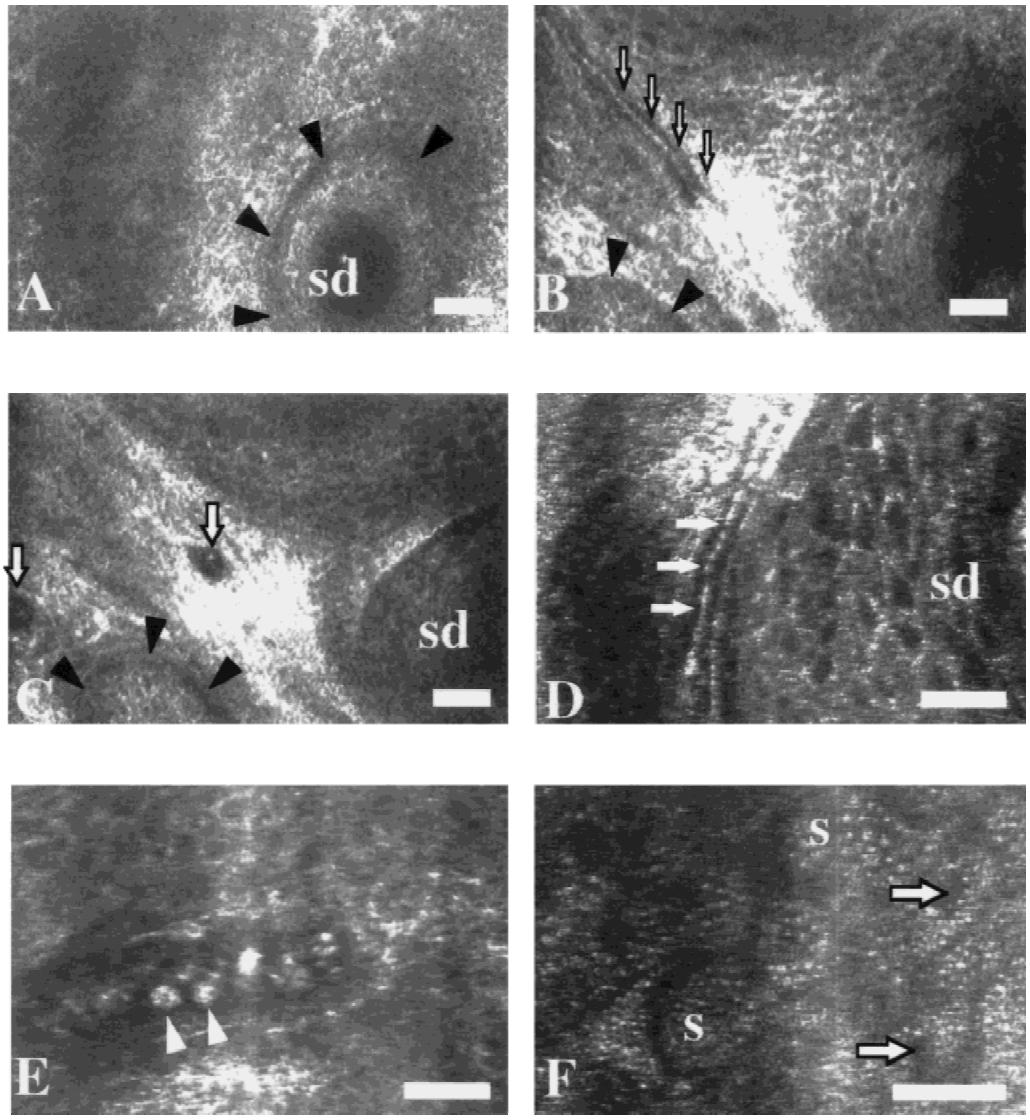

Fig. 2. Horizontal *en face* confocal images of a sebaceous gland hyperplasia lesion show hyperplastic sebaceous ducts (black arrows, sd; **A–D**) with prominent surrounding microvasculature (white arrows; **B–E**). B and C correspond to optical sections from two different depths of same skin site (70 and 95 μm below stratum corneum, respectively). In vivo confocal imaging allows high-resolution visualization of dynamic blood flow (white arrows; B,C), where delineation of individual peripheral blood cells (white arrowheads) in the supporting microvasculature is possible (E). Intracellular detail is seen in in vivo sebocytes (s; **F**) where dark nuclei (white arrows) surrounded by bright multilobular cytoplasm were seen from 180 μm depth. Unstained in vivo sections. Scale bars = 40 μm, 60×, 0.85 N.A. water immersion objective lens in A–C; 25 μm, 100×, 1.2 N.A. water immersion objective lens in D–F.

imaged in its native state as many times as required. In 1995, our laboratory reported the ability to image nuclear- and cellular-level detail in human skin in vivo with a video-rate confocal scanning laser microscope [5]. We have recently improved image quality in terms of resolution and contrast, by using a near-infrared low power Nd:YAG (1,064 nm) laser. Longer near-infrared wavelengths allow deeper imaging compared to shorter visible wavelengths [5]. Although the resolution decreases as the wavelength increases, we can image with acceptable resolution to see cellular and nuclear detail. Using water immersion objective lenses of numerical aperture (NA) 0.85–1.2, we can image transverse (en face) sections of skin with (measured) thickness of 3–5 μm, and (measured) lateral resolution of 0.5–1 μm, to a depth of 350 μm below stratum corneum

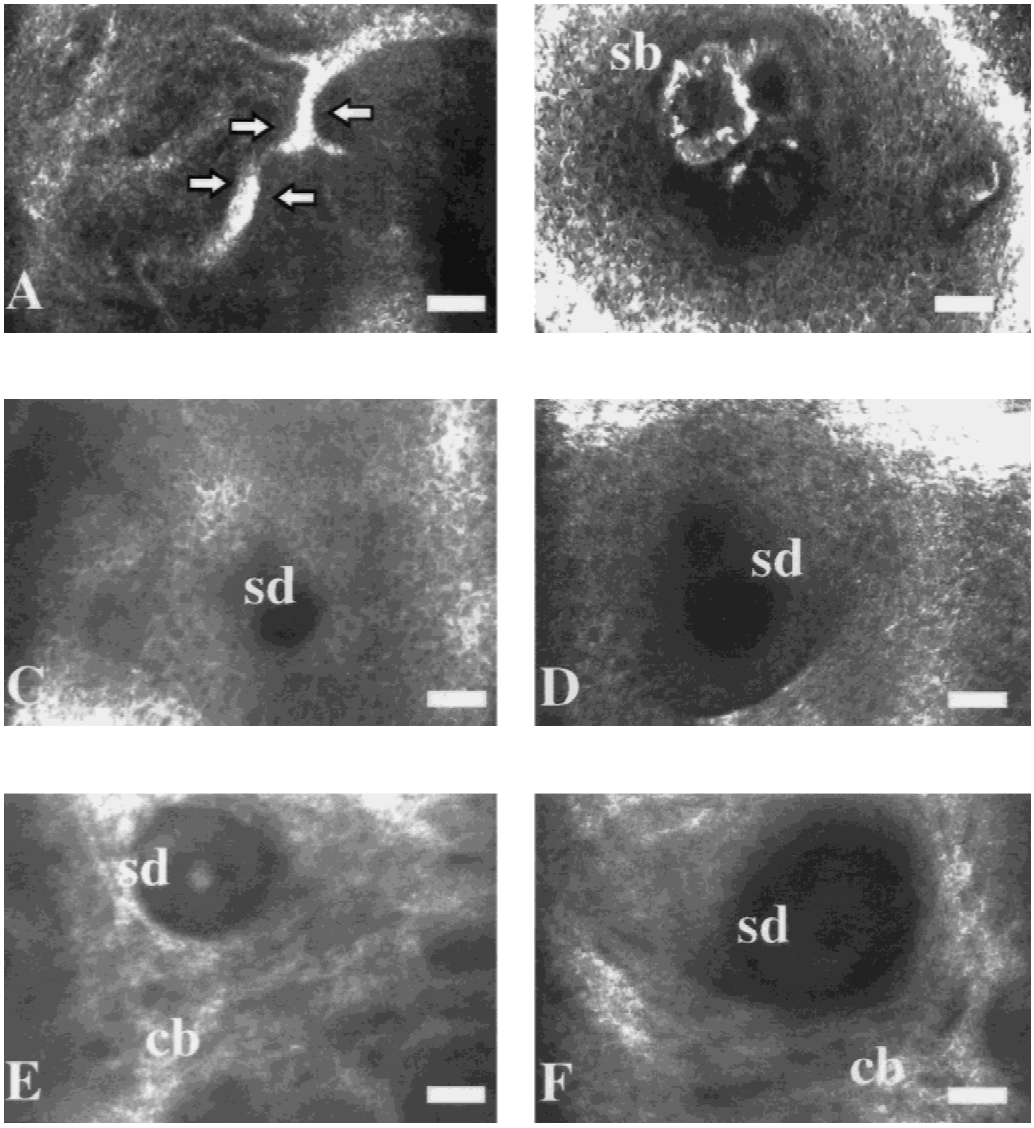

Fig. 3. Horizontal en face confocal images of sebaceous gland hyperplasia lesion after 585 nm PDL treatment. Noninvasive, real time confocal imaging immediately after PDL treatment showed selective targeting of the vasculature by the presence of photocoagulated blood vessels (white arrows; **A**). A progressive smoothing of the contours of the nonhyperplastic sebaceous duct (sd) was seen at two weeks and two months after PDL treatment (**B–F**). Presence of sebum (sb) at the top of the duct was also evidenced (B). Confocal imaging at deeper penetration showed dense collagen bundles without significant vasculature support (E and F). Unstained in vivo sections. Scale bars = 40 μm, 60×, 0.85 N.A. water immersion objective lens in A, B, and D; 80 μm, 30×, 0.9 N.A. water immersion objective lens in C, E, and F.

[6]. Confocal imaging of skin at the dermo-epidermal level can define the histologic margin of a proliferative skin lesion in vivo, such as psoriasis, based on morphometric analysis of size and number of dermal papillae as well as enlargement of blood vessels within the lesion [7].

In vivo CM, therefore, allows the study, non-invasively, of the events that might explain the involution of these lesions after laser therapy. Selective photothermal damage was confined to the supporting microvasculature, followed by decreased epidermal hyperplasia within the ductal infundibulum, and a profound reduction in the size of the hypertrophic sebaceous gland, including undifferentiated sebocytes [8]. After treatment we were unable to visualize the sebaceous gland, probably due to a deeper location in the viable skin.

In conclusion, PDL may be an alternative treatment for sebaceous gland hyperplasia. Furthermore, real time CM allows the visualization of its dynamic pathophysiologic process overtime

and may potentially be useful to study mechanisms and assess efficacy of laser (and nonlaser) therapy in other dermatologic conditions.

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